

In the Specification:

Submitted herewith is a substitute Specification identical to that which was submitted in the parent application, except that in the substitute Specification submitted herewith, line numbering has been removed and paragraph numbering has been added.

Please replace paragraph [0001] and substitute therefor paragraph [0001] submitted in clean, replacement form herein below.

**[0001]** This application is a continuation of U.S. Serial No. 09/469,200, filed December 21, 1999, entitled "HYALURONATE SYNTHASE GENE AND USES THEREOF", which is a continuation-in-part of U.S. Serial No. 08/899,040, filed July 23, 1997, entitled "HYALURONATE SYNTHASE GENE AND USES THEREOF", now abandoned, and which also claims the benefit of U.S. Provisional Application U.S. Serial No. 60/064,435, filed October 31, 1997, entitled "GROUP C HYALURONAN SYNTHASE GENE AND USES THEREOF".

In the Claims:

Please cancel claims 1-10, 15-38 and 42-59.

Please amend claims 11 and 39 as follows:

11. A recombinant host cell, wherein the recombinant host cell is a *Bacillus subtilis* cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase.

39. A method for producing hyaluronic acid, comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and

recovering the secreted hyaluronic acid.

Please add new claims 60-312.

60. The recombinant host cell of claim 11, wherein the purified nucleic acid segment encodes a Group A hyaluronan synthase.

61. The recombinant host cell of claim 60, wherein the host cell produces hyaluronic acid.

62. The recombinant host cell of claim 11, wherein the coding region encoding enzymatically active hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

63. The recombinant host cell of claim 11, wherein the *Bacillus subtilis* cell is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

64. The recombinant host cell of claim 63, wherein the purified nucleic acid segment encodes the *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

65. The recombinant host cell of claim 63, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1.

66. The recombinant host cell of claim 65, wherein the host cell produces hyaluronic acid.

67. The recombinant host cell of claim 63, wherein the purified nucleic acid segment encodes the Group A hyaluronan synthase.

68. The recombinant host cell of claim 67, wherein the host cell produces hyaluronic acid.

69. The recombinant host cell of claim 63, wherein the coding region encoding enzymatically active hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

70. The method of claim 39, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain further includes introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

71. The method according to claim 70, wherein the step of recovering the hyaluronic acid comprises extracting the secreted hyaluronic acid from the medium.

72. The method according to claim 71, further comprising the step of purifying the extracted hyaluronic acid.

73. The method of claim 39, wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the coding region encoding enzymatically active hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

74. A method for producing hyaluronic acid, comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain;

introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase into the *Bacillus subtilis* strain;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and

recovering the secreted hyaluronic acid.

75. The method according to claim 74, wherein the step of recovering the hyaluronic acid comprises extracting the secreted hyaluronic acid from the medium.

76. The method according to claim 74, further comprising the step of purifying the extracted hyaluronic acid.

77. The method of claim 74 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the coding region encoding enzymatically active hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

78. A recombinant host cell, wherein the recombinant host cell is a *Bacillus*

*subtilis* cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

79. The recombinant host cell of claim 78, wherein the host cell produces hyaluronic acid.

80. The recombinant host cell of claim 78, wherein the coding region encoding enzymatically active Group C hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

81. The recombinant host cell of claim 78, wherein the *Bacillus subtilis* cell is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

82. A recombinant host cell, wherein the recombinant host cell is a *Bacillus subtilis* cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

83. The recombinant host cell of claim 82, wherein the host cell produces hyaluronic acid.



segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

90. A method for producing hyaluronic acid, comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and

recovering the secreted hyaluronic acid.

91. The method according to claim 90, wherein the step of recovering the hyaluronic acid comprises extracting the secreted hyaluronic acid from the medium.

92. The method according to claim 90, further comprising the step of purifying the extracted hyaluronic acid.

93. The method according to claim 90, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain further includes introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.



94. The method according to claim 90 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the coding region encoding enzymatically active Group C hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

95. The method according to claim 90, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transforming a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

96. The method according to claim 90, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transfecting a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

97. The method according to claim 90, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid

segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

98. The method according to claim 90, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

99. A method for producing hyaluronic acid, comprising the steps of:  
introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain;  
growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and  
recovering the secreted hyaluronic acid.

100. The method according to claim 99, wherein the step of recovering the hyaluronic acid comprises extracting the secreted hyaluronic acid from the medium.

101. The method according to claim 99, further comprising the step of purifying the extracted hyaluronic acid.

103. The method according to claim 99 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

104. The method according to claim 99, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transforming a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

105. The method according to claim 99, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically

active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transfecting a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

106. The method according to claim 99, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

107. The method according to claim 99, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

108. A method for producing hyaluronic acid, comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and  
recovering the secreted hyaluronic acid.

109. The method according to claim 108, wherein the step of recovering the hyaluronic acid comprises extracting the secreted hyaluronic acid from the medium.

110. The method according to claim 108, further comprising the step of purifying the extracted hyaluronic acid.

111. The method according to claim 108, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain further includes introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

112. The method according to claim 108 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain, the coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

113. The method according to claim 108, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transforming a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

114. The method according to claim 108, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transfecting a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

115. The method according to claim 108, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

116. The method according to claim 108, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis*

strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase.

117. A recombinant host cell, wherein the recombinant host cell is a *Bacillus subtilis* cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase and a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

118. The recombinant host cell of claim 117, wherein the host cell produces hyaluronic acid.

119. The recombinant host cell of claim 117, wherein the coding region encoding enzymatically active Group C hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

120. A recombinant host cell, wherein the recombinant host cell is a *Bacillus subtilis* cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 and a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

121. The recombinant host cell of claim 120, wherein the host cell produces hyaluronic acid.

122. The recombinant host cell of claim 120, wherein the coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

123. A recombinant host cell, wherein the recombinant host cell is a *Bacillus subtilis* cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase and a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase, wherein the purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase comprises a nucleotide sequence in accordance with SEQ ID NO:1.

124. The recombinant host cell of claim 123, wherein the host cell produces hyaluronic acid.

125. The recombinant host cell of claim 123, wherein the coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis*



promoter.

126. A method for producing hyaluronic acid, comprising the steps of:

- introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain;
- introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase into the *Bacillus subtilis* strain;
- growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and
- recovering the secreted hyaluronic acid.

127. The method according to claim 126, wherein the step of recovering the hyaluronic acid comprises extracting the secreted hyaluronic acid from the medium.

128. The method according to claim 126, further comprising the step of purifying the extracted hyaluronic acid.

129. The method according to claim 126 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the coding region encoding enzymatically active Group C hyaluronan synthase of the

purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

130. The method according to claim 126, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transforming a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

131. The method according to claim 126, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transfecting a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

132. The method according to claim 126, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

133. The method according to claim 126, wherein the step of introducing a

purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

134. A method for producing hyaluronic acid, comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain;

introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase into the *Bacillus subtilis* strain;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and

recovering the secreted hyaluronic acid.

135. The method according to claim 134, wherein the step of recovering the hyaluronic acid comprises extracting the secreted hyaluronic acid from the medium.

136. The method according to claim 134, further comprising the step of purifying the extracted hyaluronic acid.



a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

141. The method according to claim 134 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

142. A method for producing hyaluronic acid, comprising the steps of:

- introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1;
- introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase into the *Bacillus subtilis* strain;





*Bacillus subtilis* strain;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and

recovering the secreted hyaluronic acid.

151. Hyaluronic acid prepared by the process according to claim 150, wherein the step of growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid is further defined as growing the *Bacillus subtilis* strain in Spizizens Minimal Media plus glucose and trace elements at about 32°C to secrete hyaluronic acid.

152. Hyaluronic acid prepared by the process according to claim 150, wherein the step of growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid is further defined as growing the *Bacillus subtilis* strain in a medium containing glucose and at least one of N-acetylglucosamine and glucosamine to secrete hyaluronic acid.

153. Hyaluronic acid prepared by the process according to claim 150, wherein the step of recovering the secreted hyaluronic acid is further defined as separating the hyaluronic acid from cells and debris by filtering and then separating the hyaluronic acid from the medium by precipitation by a precipitation agent.

154. Hyaluronic acid prepared by the process according to claim 153, wherein the step of separating the hyaluronic acid from cells and debris by filtering



further includes the addition of trichloroacetic acid, which facilitates in separating cells and debris from the hyaluronic acid.

155. Hyaluronic acid prepared by the process according to claim 153, wherein the precipitation agent is an alcohol or an organic solvent.

156. Hyaluronic acid prepared by the process according to claim 155, wherein the precipitation agent is selected from the group consisting of ethanol, acetone or cetyl pyridinium chloride.

157. Hyaluronic acid prepared by the process according to claim 150, further comprising the step of purifying the extracted hyaluronic acid.

158. Hyaluronic acid prepared by the process according to claim 150, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain further includes the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

159. Hyaluronic acid prepared by the process according to claim 150 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the coding region encoding enzymatically active Group C

hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

160. Hyaluronic acid prepared by the process according to claim 150, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transforming a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

161. Hyaluronic acid prepared by the process according to claim 150, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transfecting a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

162. Hyaluronic acid prepared by the process according to claim 150, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

163. Hyaluronic acid prepared by the process according to claim 150, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase.

164. Hyaluronic acid prepared by a process comprising the steps of:  
introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain;  
growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and  
recovering the secreted hyaluronic acid.

165. Hyaluronic acid prepared by the process according to claim 164, wherein the step of growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid is further defined as growing the *Bacillus subtilis* strain in Spizizens Minimal Media plus glucose and trace elements at about 32°C to secrete hyaluronic acid.

166. Hyaluronic acid prepared by the process according to claim 164, wherein the step of growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid is further defined as growing the *Bacillus subtilis* strain in a medium containing glucose and at least one of N-acetylglucosamine and glucosamine to

secrete hyaluronic acid.

167. Hyaluronic acid prepared by the process according to claim 164, wherein the step of recovering the secreted hyaluronic acid is further defined as separating the hyaluronic acid from cells and debris by filtering and then separating the hyaluronic acid from the medium by precipitation by a precipitation agent.

168. Hyaluronic acid prepared by the process according to claim 167, wherein the step of separating the hyaluronic acid from cells and debris by filtering further includes the addition of trichloroacetic acid, which facilitates in separating cells and debris from the hyaluronic acid.

169. Hyaluronic acid prepared by the process according to claim 167, wherein the precipitation agent is an alcohol or an organic solvent.

170. Hyaluronic acid prepared by the process according to claim 169, wherein the precipitation agent is selected from the group consisting of ethanol, acetone or acetyl pyridinium chloride.

171. Hyaluronic acid prepared by the process according to claim 164, further comprising the step of purifying the extracted hyaluronic acid.

172. Hyaluronic acid prepared by the process according to claim 164 wherein,

in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain further includes the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

173. Hyaluronic acid prepared by the process according to claim 164 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

174. Hyaluronic acid prepared by the process according to claim 164 wherein, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as transforming a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

175. Hyaluronic acid prepared by the process according to claim 164, wherein the step of introducing a purified nucleic acid segment having a coding region

encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as transfecting a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

176. Hyaluronic acid prepared by the process according to claim 164, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

177. Hyaluronic acid prepared by the process according to claim 164, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

178. Hyaluronic acid prepared by a process comprising the steps of:  
introducing a purified nucleic acid segment having a coding region

encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and  
recovering the secreted hyaluronic acid.

179. Hyaluronic acid prepared by the process according to claim 178, wherein the step of growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid is further defined as growing the *Bacillus subtilis* strain in Spizizens Minimal Media plus glucose and trace elements at about 32°C to secrete hyaluronic acid.

180. Hyaluronic acid prepared by the process according to claim 178, wherein the step of growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid is further defined as growing the *Bacillus subtilis* strain in a medium containing glucose and at least one of N-acetylglucosamine and glucosamine to secrete hyaluronic acid.

181. Hyaluronic acid prepared by the process according to claim 178, wherein the step of recovering the secreted hyaluronic acid is further defined as separating the hyaluronic acid from cells and debris by filtering and then separating the hyaluronic acid from the medium by precipitation by a precipitation agent.

182. Hyaluronic acid prepared by the process according to claim 181, wherein the step of separating the hyaluronic acid from cells and debris by filtering further includes the addition of trichloroacetic acid, which facilitates in separating cells and debris from the hyaluronic acid.

183. Hyaluronic acid prepared by the process according to claim 181, wherein the precipitation agent is an alcohol or an organic solvent.

184. Hyaluronic acid prepared by the process according to claim 183, wherein the precipitation agent is selected from the group consisting of ethanol, acetone or cetyl pyridinium chloride.

185. Hyaluronic acid prepared by the process according to claim 178, further comprising the step of purifying the extracted hyaluronic acid.

186. Hyaluronic acid prepared by the process according to claim 178, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain further includes the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active UDP-glucose dehydrogenase.

187. Hyaluronic acid prepared by the process according to claim 178 wherein, in the step of introducing a purified nucleic acid segment having a coding region



encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain, the coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of the purified nucleic acid segment is under control of a *Bacillus subtilis* promoter.

188. Hyaluronic acid prepared by the process according to claim 178, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as transforming a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

189. Hyaluronic acid prepared by the process according to claim 178, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as transfecting a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

190. Hyaluronic acid prepared by the process according to claim 178, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase

of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as transducing a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

191. Hyaluronic acid prepared by the process according to claim 178 wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain is further defined as electroporating a *Bacillus subtilis* strain with a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2.

192. A recombinant host cell wherein the recombinant host cell is a *Bacillus subtilis* cell having an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc, the recombinant host cell further transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an enzymatically active hyaluronan synthase.

193. The recombinant host cell of claim 192, wherein the recombinant host cell further includes at least one mutated RNA polymerase promoter capable of expressing RNA polymerase in an amount greater than an endogenous RNA polymerase promoter.

194. The recombinant host cell of claim 192, wherein the recombinant host cell is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme.

195. The recombinant host cell of claim 194, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

196. The recombinant host cell of claim 194, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

197. The recombinant host cell of claim 192, wherein the recombinant host cell further includes at least one additional messenger RNA stabilizing element than is found in a native *Bacillus subtilis* cell.

198. The recombinant host cell of claim 192, wherein the recombinant host cell further includes at least one less messenger RNA destabilizing element than is found in a native *Bacillus subtilis* cell.

199. The recombinant host cell of claim 192, wherein the recombinant host cell further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

200. The recombinant host cell of claim 192, wherein the recombinant host cell further includes at least one mutated UDP-sugar precursor gene wherein the mutated UDP-sugar precursor gene increases a half-life of a transcribed messenger RNA.

201. The recombinant host cell of claim 192, wherein the recombinant host cell further includes at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

202. The recombinant host cell of claim 201, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

203. A method for producing hyaluronic acid, comprising the steps of:  
introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, wherein the *Bacillus subtilis* strain has an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc;  
growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and  
recovering the secreted hyaluronic acid.

204. The method according to claim 203, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated RNA polymerase promoter having an increased promoter activity.

205. The method according to claim 203, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain is transformed with a vector comprising a purified nucleic acid segment having a coding region encoding an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

206. The method according to claim 205, wherein the UDP-sugar precursor biosynthesis pathway is UDP-glucose dehydrogenase.

207. The method according to claim 205, wherein the UDP-sugar precursor biosynthesis pathway is UDP-glucose pyrophosphorylase.

208. The method according to claim 203, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one additional messenger RNA stabilizing element than is found in a native *Bacillus subtilis* strain.

209. The method according to claim 203, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one less messenger RNA destabilizing element than is found in a native *Bacillus subtilis* strain.

210. The method according to claim 203, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

211. The method according to claim 203, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated UDP-sugar precursor gene wherein the mutation results in an increase of a half-life of a messenger RNA transcribed from the mutated UDP-sugar precursor gene.

212. The method according to claim 203, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis*

strain further includes at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

213. The method according to claim 212, wherein the mutation to the UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a binding affinity of a ribosome for the ribosome binding site is increased.

214. A recombinant host cell wherein the recombinant host cell is a *Bacillus subtilis* cell having an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc, the recombinant host cell further being transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an enzymatically active Group C hyaluronan synthase.

215. The recombinant host cell of claim 214, wherein the recombinant host cell further includes at least one mutated RNA polymerase promoter capable of expressing RNA polymerase in an amount greater than an endogenous RNA polymerase promoter.

216. The recombinant host cell of claim 214, wherein the recombinant host cell is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an UDP-sugar precursor biosynthesis pathway enzyme.

217. The recombinant host cell of claim 216, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

218. The recombinant host cell of claim 216, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

219. The recombinant host cell of claim 214, wherein the recombinant host cell further includes at least one additional messenger RNA stabilizing element than is found in a native *Bacillus subtilis* cell.

220. The recombinant host cell of claim 214, wherein the recombinant host cell further includes at least one less messenger RNA destabilizing element than is found in a native *Bacillus subtilis* cell

221. The recombinant host cell of claim 214, wherein the recombinant host cell further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

222. The recombinant host cell of claim 214, wherein the recombinant host cell further includes at least one mutated UDP-sugar precursor gene wherein the mutated UDP-sugar precursor gene increases a half-life of a transcribed messenger RNA.



223. The recombinant host cell of claim 214, wherein the recombinant host cell further includes at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

224. The recombinant host cell of claim 223, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

225. A recombinant host cell wherein the recombinant host cell is a *Bacillus subtilis* cell having an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc, the recombinant host cell further being transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an enzymatically active *Streptococcus equismilis* hyaluronan synthase of SEQ ID NO:2.

226. The recombinant host cell of claim 225, wherein the recombinant host cell further includes at least one mutated RNA polymerase promoter capable of expressing RNA polymerase in an amount greater than an endogenous RNA polymerase promoter.

227. The recombinant host cell of claim 225, wherein the recombinant host cell is transformed with a recombinant vector comprising a purified nucleic acid

segment having a coding region encoding an UDP-sugar precursor biosynthesis pathway enzyme.

228. The recombinant host cell of claim 227, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

229. The recombinant host cell of claim 227, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

230. The recombinant host cell of claim 225, wherein the recombinant host cell further includes at least one additional messenger RNA stabilizing element than is found in a native *Bacillus subtilis* cell.

231. The recombinant host cell of claim 225, wherein the recombinant host cell further includes at least one less messenger RNA destabilizing element than is found in a native *Bacillus subtilis* cell.

232. The recombinant host cell of claim 225, wherein the recombinant host cell further includes at least one nucleic acid segment encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

233. The recombinant host cell of claim 225, wherein the recombinant host cell further includes at least one mutated UDP-sugar precursor gene, wherein the

mutated UDP-sugar precursor gene increases a half-life of a transcribed messenger RNA.

234. The recombinant host cell of claim 225, wherein the recombinant host cell further includes at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

235. The recombinant host cell of claim 234, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

236. A recombinant host cell wherein the recombinant host cell is a *Bacillus subtilis* cell having an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc and the recombinant host cell further being transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an enzymatically active Group C hyaluronan synthase of SEQ ID NO:1.

237. The recombinant host cell of claim 236, wherein the recombinant host cell further includes at least one mutated RNA polymerase promoter capable of expressing RNA polymerase in an amount greater than an endogenous RNA polymerase promoter.

238. The recombinant host cell of claim 236, wherein the recombinant host cell is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an UDP-sugar precursor biosynthesis pathway enzyme.

239. The recombinant host cell of claim 238, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

240. The recombinant host cell of claim 238, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

241. The recombinant host cell of claim 236, wherein the recombinant host cell further includes at least one additional messenger RNA stabilizing element than is found in a native *Bacillus subtilis* cell.

242. The recombinant host cell of claim 236, wherein the recombinant host cell further includes at least one less messenger RNA destabilizing element than is found in a native *Bacillus subtilis* cell.

243. The recombinant host cell of claim 236, wherein the recombinant host cell further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.



purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated RNA polymerase promoter having an increased promoter activity.

249. The method according to claim 247, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

250. The method according to claim 249, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

251. The method according to claim 249, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

252. The method according to claim 247, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one additional messenger RNA stabilizing element than is found in the *Bacillus subtilis* cell used to form the recombinant host cell.



active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

257. The method according to claim 256, wherein the mutation to the UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a binding affinity of a ribosome for the ribosome binding site is increased.

258. A method for producing hyaluronic acid, comprising the steps of:  
introducing a purified nucleic acid segment having a coding  
region encoding enzymatically active *Streptococcus*  
*equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus*  
*subtilis* strain, wherein the *Bacillus subtilis* strain has an enhanced  
production of at least one of UDP-GlcA and UDP-GlcNAc;  
growing the *Bacillus subtilis* strain in a medium to secrete  
hyaluronic acid; and  
recovering the secreted hyaluronic acid.

259. The method according to claim 258, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated RNA polymerase promoter having an increased promoter activity.



260. The method according to claim 258, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain is transformed with a vector comprising a purified nucleic acid segment having a coding region encoding an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

261. The method according to claim 260, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

262. The method according to claim 260, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

263. The method according to claim 258, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one additional messenger RNA stabilizing element than is found in a native *Bacillus subtilis* strain.

264. The method according to claim 258, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one

less messenger RNA destabilizing element than is found in a native *Bacillus subtilis* strain.

265. The method according to claim 258, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

266. The method according to claim 258, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain comprises at least one mutated endogenous UDP-sugar precursor gene wherein the mutation results in an increase of a half-life of a messenger RNA transcribed from the mutated UDP-sugar precursor gene.

267. The method according to claim 258, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain comprises at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an

increased translational efficiency.

268. The method according to claim 267, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

269. A method for producing hyaluronic acid, comprising the steps of:  
introducing a purified nucleic acid segment having a coding  
region encoding enzymatically active *Streptococcus*  
*equismillis* hyaluronan synthase into a *Bacillus subtilis*  
strain wherein the purified nucleic acid segment comprises a  
nucleotide sequence in accordance with SEQ ID NO:1, and  
wherein the *Bacillus subtilis* strain has an enhanced production of  
at least one of UDP-GlcA and UDP-GlcNAc;  
growing the *Bacillus subtilis* strain in a medium to secrete  
hyaluronic acid; and  
recovering the secreted hyaluronic acid.

270. The method according to claim 269, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated RNA polymerase promoter having a greater promoter activity than an endogenous

endogenous RNA polymerase promoter.

271. The method according to claim 269, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

272. The method according to claim 271, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

273. The method according to claim 271, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

274. The method according to claim 269, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one additional messenger RNA stabilizing element than is found in a native *Bacillus subtilis* strain.

275. The method according to claim 269, wherein the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically

active *Streptococcus equismillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one less messenger RNA destabilizing element than is found a native *Bacillus subtilis* strain.

276. The method according to claim 269, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

277. The method according to claim 269, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated endogenous UDP-sugar precursor gene wherein the mutation results in an increase of a half-life of a messenger RNA transcribed from the mutated UDP-sugar precursor gene.

278. The method according to claim 269, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equismillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated UDP-

sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

279. The method according to claim 278, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

280. Hyaluronic acid prepared by a process comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, wherein the *Bacillus subtilis* strain has an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and

recovering the secreted hyaluronic acid.

281. Hyaluronic acid prepared by the process according to claim 280, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated RNA polymerase promoter having a greater promoter activity than an endogenous RNA polymerase promoter.

282. Hyaluronic acid prepared by the process according to claim 297, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

283. Hyaluronic acid prepared by the process according to claim 282, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

284. Hyaluronic acid prepared by the process according to claim 282, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

285. Hyaluronic acid prepared by the process according to claim 280, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one additional messenger RNA stabilizing element than is found in the *Bacillus subtilis* cell used to form the recombinant host cell.

286. Hyaluronic acid prepared by the process according to claim 280, wherein in the step of introducing a purified nucleic acid segment having a coding region

encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one less messenger RNA destabilizing element than is found in the *Bacillus subtilis* cell used to form the recombinant host cell.

287. Hyaluronic acid prepared by the process according to claim 280, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

288. Hyaluronic acid prepared by the process according to claim 280, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated UDP-sugar precursor gene wherein the mutation results in an increase of a half-life of a messenger RNA transcribed from the mutated UDP-sugar precursor gene.

289. Hyaluronic acid prepared by the process according to claim 280, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active Group C hyaluronan synthase into a *Bacillus*



*subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

290. Hyaluronic acid prepared by the process according to claim 289, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

291. Hyaluronic acid prepared by a process comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, wherein the *Bacillus subtilis* strain has an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc;

growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and

recovering the secreted hyaluronic acid.

292. Hyaluronic acid prepared by the process according to claim 291, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated RNA polymerase promoter having a greater

promoter activity than an endogenous RNA polymerase promoter.

293. Hyaluronic acid prepared by the process according to claim 291, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

294. Hyaluronic acid prepared by the process according to claim 293, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

295. Hyaluronic acid prepared by the process according to claim 293, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

296. Hyaluronic acid prepared by the process according to claim 291, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one additional messenger RNA stabilizing element than is

found in the *Bacillus subtilis* cell used to form the recombinant host cell.

297. Hyaluronic acid prepared by the process according to claim 291, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one less messenger RNA destabilizing element than is found in the *Bacillus subtilis* cell used to form the recombinant host cell.

298. Hyaluronic acid prepared by the process according to claim 291, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one nucleic acid segment having a coding region encoding a UDP-sugar precursor biosynthesis pathway enzyme having an activity greater than an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

299. Hyaluronic acid prepared by the process according to claim 291, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain comprises at least one mutated UDP-sugar precursor gene wherein the mutation results in an increase of a half-life of a messenger RNA transcribed from the mutated UDP-sugar precursor gene.

300. Hyaluronic acid prepared by the process according to claim 291, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase of SEQ ID NO:2 into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain comprises at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

301. Hyaluronic acid prepared by the process according to claim 300, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

302. Hyaluronic acid prepared by a process for producing hyaluronic acid, comprising the steps of:

introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1, and wherein the *Bacillus subtilis* strain has an enhanced production of at least one of UDP-GlcA and UDP-GlcNAc; growing the *Bacillus subtilis* strain in a medium to secrete hyaluronic acid; and recovering the secreted hyaluronic acid.

303. Hyaluronic acid prepared by the process according to claim 302, in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated RNA polymerase promoter having a greater promoter activity than an endogenous RNA polymerase.

304. Hyaluronic acid prepared by the process according to claim 302, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimillis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain is transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding an endogenous UDP-sugar precursor biosynthesis pathway enzyme.

305. Hyaluronic acid prepared by the process according to claim 304, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose dehydrogenase.

306. Hyaluronic acid prepared by the process according to claim 304, wherein the UDP-sugar precursor biosynthesis pathway enzyme is UDP-glucose pyrophosphorylase.

307. Hyaluronic acid prepared by the process according to claim 302, wherein



encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated endogenous UDP-sugar precursor gene wherein the mutation results in an increase of a half-life of a messenger RNA transcribed from the mutated UDP-sugar precursor gene.

311. Hyaluronic acid prepared by the process according to claim 302, wherein in the step of introducing a purified nucleic acid segment having a coding region encoding enzymatically active *Streptococcus equisimilis* hyaluronan synthase into a *Bacillus subtilis* strain, the *Bacillus subtilis* strain further includes at least one mutated UDP-sugar precursor gene encoding a messenger RNA having an increased translational efficiency.

312. Hyaluronic acid prepared by the process according to claim 311, wherein the mutated UDP-sugar precursor gene occurs in a ribosome binding site in the UDP-sugar precursor gene such that a ribosome has an increased binding affinity for the ribosome binding site.

## **REMARKS**

This amendment is submitted prior to the first examination and action of the United States Patent and Trademark Office. The claims pending in this application are amended claims 11-14 and 39-41 and newly added claims 60-312. It is Applicant's belief that the inventive concept recited in such claims is patentable over the art of record in the parent application and that such claims are necessary to afford Applicant with the degree of patent protection to which Applicant is entitled by law.

Should the Examiner have any questions or comments concerning the before-mentioned amendments to the application or any other matter, Applicant's attorney will welcome the opportunity to discuss same with the Examiner.

Respectfully submitted,



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## ATTACHMENT A

### In the Specification:

[0001] This application is a continuation of U.S. Serial No. 09/469,200, filed December 21, 1999, entitled "HYALURONATE SYNTHASE GENE AND USES THEREOF", which is a continuation-in-part of U.S. Serial No. 08/899,040, filed July 23, 1997, entitled "HYALURONATE SYNTHASE GENE AND USES THEREOF", now abandoned, and **[relates to]** which also claims the benefit of U.S. Provisional Application U.S. Serial No. 60/064,435, filed October 31, 1997, entitled "GROUP C HYALURONAN SYNTHASE GENE AND USES THEREOF".

### In the Claims:

11. (Once Amended) A recombinant host cell, wherein the recombinant host cell is a **[prokaryotic]** *Bacillus subtilis* cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase.
39. (Once Amended) A method for producing hyaluronic acid, comprising the steps of:
- introducing a purified nucleic acid segment having a coding region encoding enzymatically active hyaluronan synthase into a **[host organism, wherein the host organism contains nucleic**

